John von Neumann Institute for Computing

Transition Metal Centers in Biological Matrices: Why Nature Has Chosen Vanadate as Cofactor for Haloperoxidase

D. Geibig, R. Wilcken, M. Bangesh, W. Plass

published in

NIC Symposium 2008,
G. Münster, D. Wolf, M. Kremer (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **39**, ISBN 978-3-9810843-5-1, pp. 71-78, 2008.

http://www.fz-juelich.de/nic-series/volume39

Transition Metal Centers in Biological Matrices: Why Nature Has Chosen Vanadate as Cofactor for Haloperoxidase

Daniel Geibig, Rainer Wilcken, Masroor Bangesh, and Winfried Plass

Institut für Anorganische und Analytische Chemie, Lehrstuhl für Anorganische Chemie II
Friedrich-Schiller-Universität Jena, 07743 Jena, Germany
E-mail: sekr.plass@uni-jena.de

While molybdate(VI) shows superior catalytic activity towards halide oxidation in solution as compared to its close chemical relative vanadate(V), the vanadium haloperoxidase enzymes nevertheless employ the latter as cofactor. How can the enormous effectiveness of the enzyme be explained? What are the details of the reaction mechanism? What are the chemical features differentiating relevant vanadium and molybdenum species? These are the questions that we posed in our computational DFT studies on several model systems of haloperoxidases. We are able to postulate an enzymatic mechanism and to explain why there are no molybdenum haloperoxidase enzymes.

1 Introduction and Motivation

The need to save energy wherever possible dominates today's life. In chemistry, the efforts in this direction have been summarized by the term "Green Chemistry". In order to produce basic chemical compounds, processes consuming a lot of energy are often necessary. Using catalysts, energy consumption can be lowered by a great amount. However, developing new catalytic compounds is by no means an easy task.

Nature uses very effective and complex catalysts – the enzymes. Imitating nature in order to develop catalysts working in a similar way to enzymes is thus an important research area of both chemists and biochemists alike. To achieve this goal, it is necessary to first elucidate the underlying catalytic mechanism. Using computational chemistry and employing suitable model systems, it is possible to study chemical reactions at the molecular level. In this project, the enzyme family of vanadium haloperoxidases (VHPOs) is investigated.

This group of enzymes is found in several seaweeds as well as fungi and plays an important role synthesizing halogenated organic compounds in natural environments. VHPOs catalyze the two-electron oxidation of a halide ion by hydrogen peroxide yielding hypohalous acid given in Eq. 1, which in turn is able to halogenate various organic compounds.

$$\mathrm{H_2O_2} + \mathrm{H^+} + \mathrm{X^-} \rightarrow \mathrm{H_2O} + \mathrm{HOX} \quad (1)$$

The structure of the active site in vanadium chloroperoxidase from *curvularia inaequalis* was investigated by X-ray crystallography and published by Wever et al. in 1996[1]. In subsequent years several studies were conducted to further elucidate the mechanism of the catalytic cycle[2–6]. The centre of the active site consists of a vanadate(V) ion in trigonal bipyramidal geometry, bound to His_{496} and forming hydrogen bonds to the surrounding amino acids[7] (Fig. 1). By a reaction with hydrogen peroxide and elimination of the re-

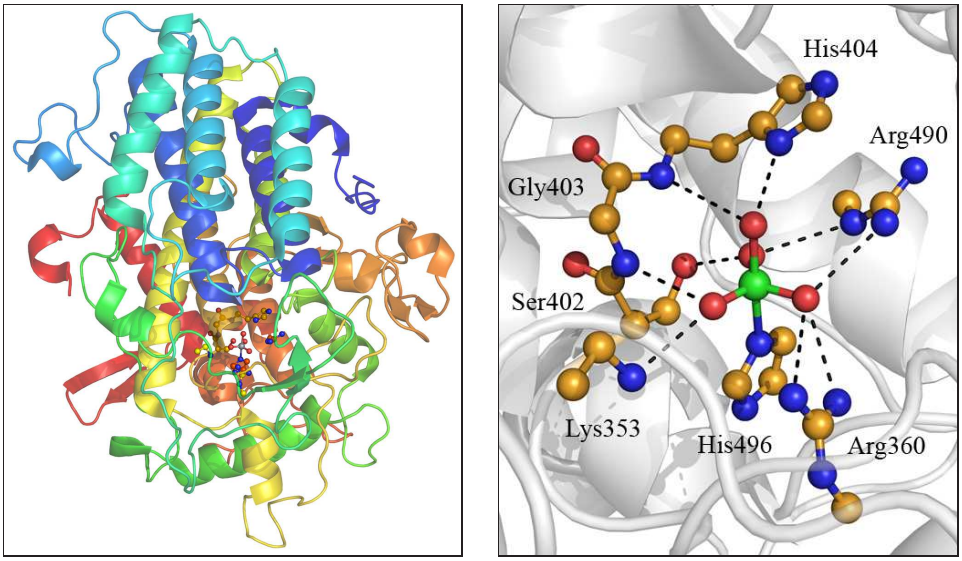

Figure 1. Left: Crystal structure of native vanadium-containing chloroperoxidase from *curvularia inaequalis* (PDB ID: 1IDQ)[3]. Right: Cutout of the active site.

sulting water molecule, a reactive peroxo species is formed (Fig. 2). Several studies have indicated that the peroxo group is activated by protonation before oxidizing the halide ion in a second step[8]. Then, the halide ion attacks the reactive oxygen atom in a nucleophilic manner and a hypohalous acid or similar "X^+" species is formed.

Due to the fact that these compounds possess versatile applicability in reactions with different nucleophilic acceptors, it is desirable to develop catalysts mimicking haloperoxidases. The simplest concept would be to employ solely the cofactor vanadate(V)[9]. However, it was found that molybdate(VI) is actually 45 times more reactive than vanadate(V), a surprising fact that was explained by the ability of molybdate(VI) to form reactive oxodiperoxo species[10–12]. Vanadate(V) is also able to form oxodiperoxo species which nevertheless are not able to oxidize bromide ions[13].

A setback to the concept of using molybdate(VI) instead of vanadate(V) as cofactor came with H. Vilter's reconstitution experiments using the apoprotein of VHPO from *ascophyllum nodosum*[14]. Replacing the vanadate cofactor with molybdate results in a total loss of catalytic activity. Taking the very similar ionic radii of V^{5+} and Mo^{6+} into account, it can however be suggested that the molybdenum cation can take the position of the vanadium cation in the enzyme.

The resulting question is: Why does the holoenzyme of vanadium bromoperoxidase not show catalytic activity when employing a molybdate cofactor, when in contrast molybdate(VI) possesses catalytic activity in solution that by far surpasses the activity of vanadate(V)? All previously made observations indicate that the reason might be differences in the catalytic mechanisms of both species. Investigations of these differences are one important goal of our work.

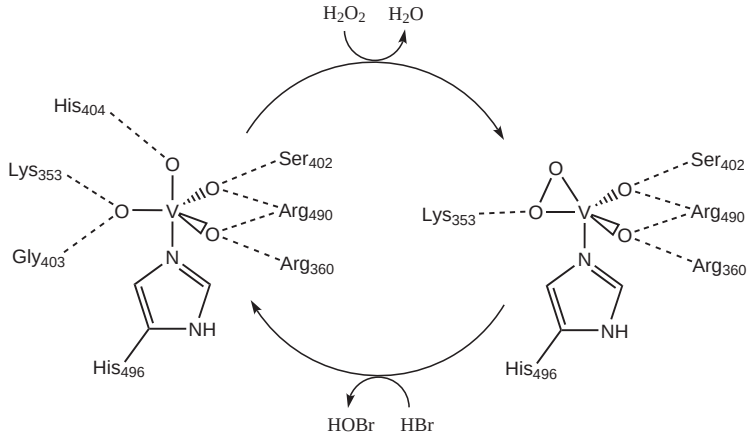

Figure 2. Scheme of the enzymatic cycle, showing the native form of the vanadium-containing chloroperoxidase (left) as well as the reactive peroxo species (right) according Ref. 3.

2 Computational Methods

It is common knowledge in quantum chemistry that density functional theory according to the Kohn-Sham scheme is well suited to investigate transition metal complexes. All DFT calculations in this work were performed using the program packages *Amsterdam Density Functional*[15–17] (ADF) and TURBOMOLE[18]. Due to the fact that the Schrödinger equation cannot be solved exactly for many-electron molecular systems, their quantum chemical treatment requires several approximations. The quality of a DFT calculation mainly depends on two factors, the nature of the exchange-correlation functional E_{xc} employed and the basis set. In this work, the gradient corrected (GGA) functional of Becke[19] and Perdew[20] (BP86) was used together with the TZVP basis set which is of split-valence triple-ζ quality with additional polarization functions on all atoms. In calculations with the ADF package, the scalar ZORA (*zero-order regular approximation*) method implemented into ADF by E. van Lenthe et al.[21] was used to account for relativistic effects of molybdenum. In the TURBOMOLE calculations, a relativistic ECP (*effective core potential*)[22] for molybdenum was used.

Massively parallelized high performance computer systems are the most important tool for computer chemists, as larger basis sets and more complex functionals can be employed in order to reach more accurate results.

Carrying out geometry optimizations of molecular structures, stationary points such as local minima and maxima on the many-dimensional potential surface can be located. A verification of the nature of intermediate structures (local minima), such as reactants and products, and transition state structures (local maxima) can be achieved by means of vibrational frequency calculations. These frequency calculations also yield thermodynamic data such as enthalpy and entropy values which allow for the construction of a Gibbs free energy profile for all reaction steps, including several competing reaction paths. The path possessing the lowest energy is the most probable path for the reaction. Nevertheless, it is

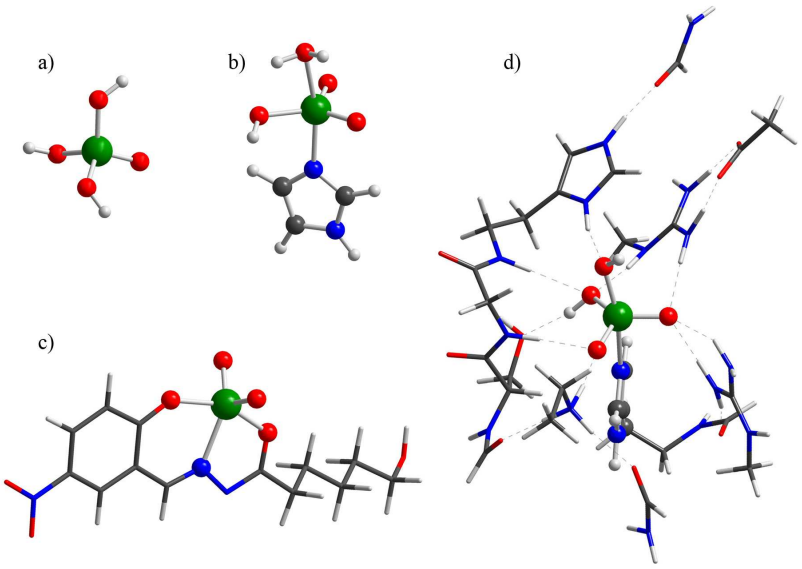

Figure 3. Several structures of model systems for investigations concerning haloperoxidase activity, optimized with TURBOMOLE/BP86(RI)/TZVP. a) vanadate(V), b) imidazole-bound vanadate(V) with apical water, c) example vanadium complex $[VO_2(NO_2salhyhh)]^-$, d) cutout of the active site of VCPO.

necessary to investigate every chemically possible reaction path, a process which consumes a lot of computational time. Using quantum chemistry, it is thus possible to understand and explain mechanisms of chemical reactions.

3 Molecular Model Systems

Even with today's arrays of high performance computer clusters, it is still impossible to treat large molecular systems like whole enzymes in a quantum chemical way. Thus molecular model systems represent a central tool to elucidate chemical reaction mechanisms. The choice of a model system has decisive influence on the results obtained. In general it can be stated that larger model systems lead to more accurate results than smaller ones. In Fig. 3 different model systems whose haloperoxidase activity was investigated in this project are shown. In addition to the vanadium species shown, the corresponding molybdenum species were also examined to uncover differences in the catalytic cycles. Additional systems with varying degrees of protonation can also be envisaged. Structures a) to c) are very simple model systems sharing only basic similarity with the active site of VHPO. Their advantage lies in the fact that they are made up of a small number of atoms, requiring less computational time than system d) and thus allowing the investigation of numerous possible reaction paths. Interpreting the results obtained in a reasonable way, plenty of information regarding the catalytic cycle of the enzyme can be obtained.

Model system d) represents a cutout of the active site of vanadium chloroperoxidase. Solely in this model system, the important influence of the hydrogen bonds formed by vanadate and the surrounding amino acid is considered. It thus constitutes the best model

system but introduces additional problems, apart from the dramatically increased computational demands: The positions of a total of 26 atoms of the protein backbone must be fixed in order to prevent the molecular system – which is now not held together anymore by the enzyme matrix – from falling apart and to preserve the active site structure.

4 Catalytic Mechanism of the Enzyme

Many open questions in connection with the catalytic mechanism of the VHPOs were answered by extensive theoretical studies in recent years, and finally an enzymatic cycle, shown in Fig. 4, has been postulated[5,23]. As can easily be seen, the amino acids bound to the cofactor by hydrogen bonds play an important role. In the first reaction step, the protonated His_{404} delivers its proton to the axial hydroxo group of the cofactor, forming a water molecule (4b). This water molecule can now be replaced by hydrogen peroxide in a dissociative manner, which then leads to the catalytically active peroxo species after the elimination of an additional water molecule (4c-d). In the second step of the catalytic reaction, Lys_{353} activates the peroxo group of the cofactor by means of hydrogen bonding, in this way facilitating the halide oxidation process (4e-f).

These results were obtained using the model system b) of Fig. 3 selectively expanded by individual amino acid residues. Due to limited computational resources, only selected

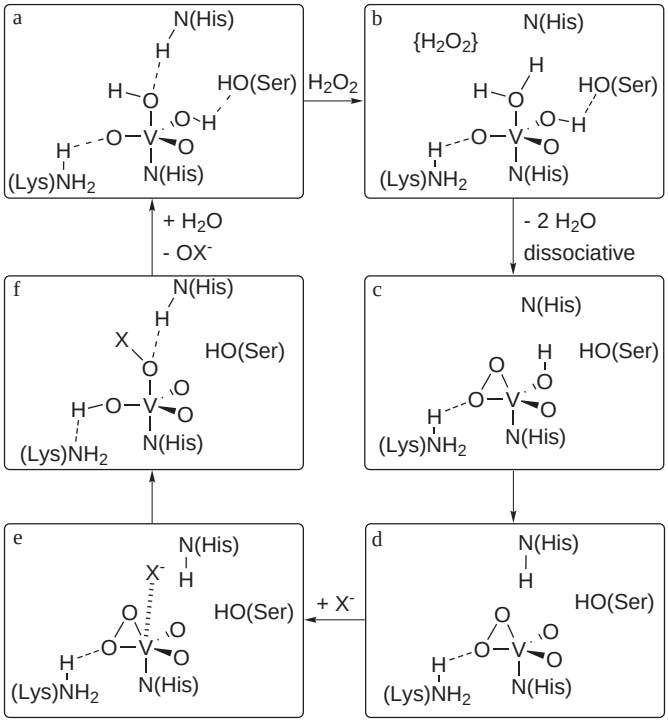

Figure 4. Proposed catalytic cycle for the halide oxidation by vanadium-containing haloperoxidases[23]; emphasizing the specific role of the amino acid residues Lys_{353}, Ser_{402} and His_{404} (see Fig. 1).

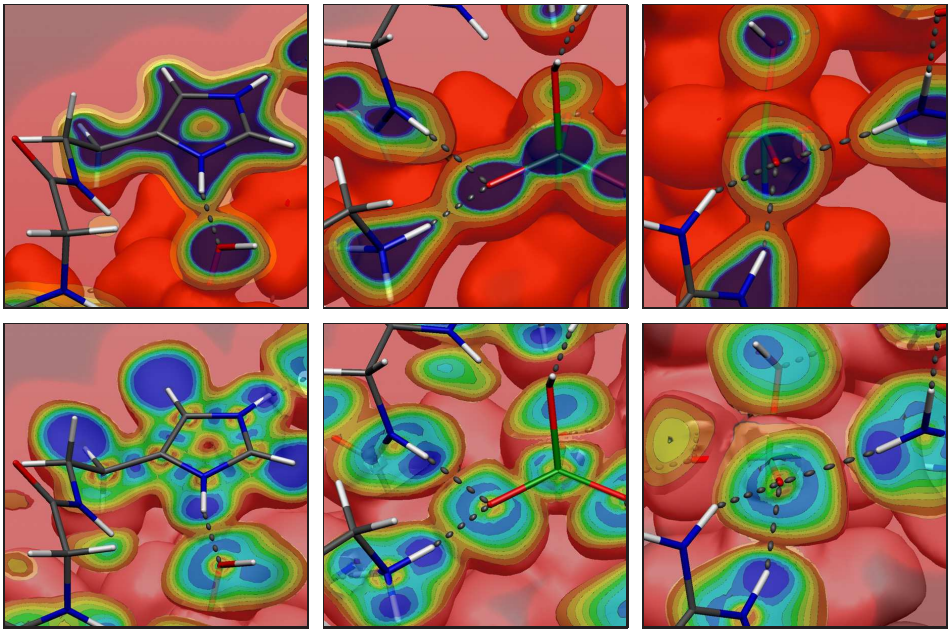

Figure 5. Plot of the electron density (first row) as well as a plot of the electron localization function (second row), highlighting selected hydrogen bonds. From left to right: His_{404}–$\mathrm{H}\cdots\mathrm{OH}_{ax}$, Lys_{353}–$\mathrm{H}\cdots\mathrm{O}_{eq}$ and Arg_{360}–$\mathrm{H}\cdots\mathrm{O}_{eq}$.

results could be verified using model system d). Investigations of the hydrogen bonding network in the native structure of the active site (model system d) by electron density and electron localization function (ELF) calculations (shown in Fig. 5) yielded particularly interesting results. Emphasis in further computational studies will be laid on an investigation of changes in the hydrogen bonding network during the course of the catalytic reaction.

5 Difference between Vanadium and Molybdenum Species

In order to understand why nature prefers vanadate(V) instead of choosing the catalytically more active molybdate(VI) as cofactor for haloperoxidases, DFT calculations on molybdate model systems similar to b) in Fig. 3 were performed. The results obtained show that the oxodiperoxo species $[\mathrm{MoO(O_2)_2Im}]$ is indeed more catalytically active than the dioxo-monoperoxo species $[\mathrm{MoO_2(O_2)Im}]$. However, the spatial environment of the VHPO active site is tailored to a monoperoxo species. Calculations investigating the halide oxidation activity of the molybdenum(VI) monoperoxo species allow the conclusion that $[\mathrm{MoO_2(O_2)Im}]$ exhibits some catalytic activity – a contradiction to the results obtained by Vilter's studies[14] of the VHPO holoprotein. Presumably, the actual reason for the inactivity of "molybdenum bromoperoxidase" might be that no peroxo species is formed in the first place.

Ongoing studies hence explore the formation of the molybdenum monoperoxo species. The first and central step of the dissociative mechanism is the cleavage of water (Fig. 4b) and the subsequent attack by hydrogen peroxide. Since molybdenum generally supports

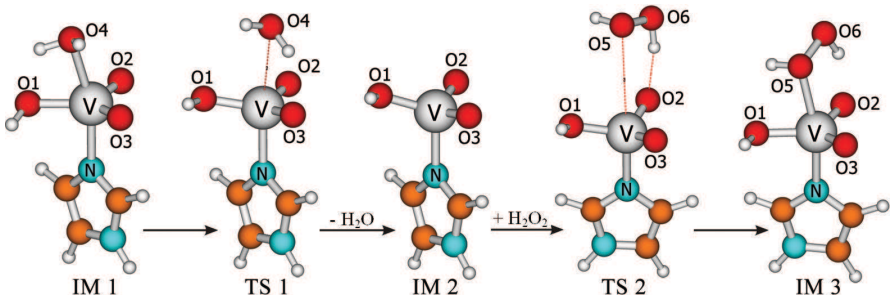

Figure 6. Intermediate and transition state structures for the reaction of hydrogen peroxide with imidazole-bound vanadate(V) optimized at ADF/BP86/TZ2P level. Analogous calculations have been done on imidazole bounded molybdate(VI).

larger coordination numbers compared to vanadium, it can be assumed that it prefers an associative reaction mechanism while vanadium prefers a dissociative one. In order to verify this claim, structures of several stationary points on the potential surface along the reaction coordinate for this first dissociative step of the catalytic cycle were calculated for both the vanadium and the molybdenum species (Fig. 6). Activation energy barriers and Gibbs free reaction energy values for both species were calculated from the free energy values of the optimized structures. Indeed an important difference is obtained: The reaction of hydrogen peroxide with $[VO_2(OH)(H_2O)Im]$ is an *exergonic* process while the same reaction employing $[MoO_2(OH)(H_2O)Im]^+$ as substrate is *endergonic*.

$$\textbf{IM1}(\mathrm{V}) + \mathrm{H_2O_2} \rightleftharpoons \textbf{IM3}(\mathrm{V}) + \mathrm{H_2O} \qquad \triangle_{\mathrm{R}}\mathrm{G} = -18,6\ kJ/mol \qquad (2)$$

$$\textbf{IM1}(\mathrm{Mo}) + \mathrm{H_2O_2} \rightleftharpoons \textbf{IM3}(\mathrm{Mo}) + \mathrm{H_2O} \qquad \triangle_{\mathrm{R}}\mathrm{G} = +24,0\ kJ/mol \qquad (3)$$

Without the formation of a peroxo complex, the catalytic reaction at the metal centre cannot proceed. This is the reason why molybdate does not show catalytic activity when embedded into the VHPO apoenzyme

6 Future Prospects

Investigations of the enzymatic cycle of vanadium haloperoxidases (Fig. 4) have so far been conducted mainly by calculations employing the small model system (Fig. 3 b). Expanding the system by the surrounding enzyme matrix (Fig. 3 d), the important hydrogen bonding network between the cofactor and several amino acid residues can be properly taken into account. Some previously obtained results can be verified using this larger model system. The TURBOMOLE suite of programs[18], now available for use on the NIC cluster JUMP, is especially well suited for these calculations on large systems as it contains the *multipole acceleration for the resolution of identity* method (MARIJ).

Calculations merging quantum chemical methods and molecular mechanics (QM/MM) represent one further step to improve the model system. MM calculations require only a fraction of the computational time needed for QM calculations. Using this approach, it is possible to model the full enzyme matrix (with MM) instead of only a small section at the active site, and thus no atoms of the backbone need to be fixed. The surrounding area of the active site is still treated in a fully quantum chemical way.

Additionally, it is desirable to take a closer look at the associative mechanism of both molybdate(VI) and vanadate(V) species, thus gaining knowledge to specifically improve ligands in order to produce molybdenum(VI) complexes exhibiting strong haloperoxidase activity.

Acknowledgments

This project is supported by a grant of computer time of the John von Neumann Institute for Computing (NIC) and a research grant of the Deutsche Forschungsgemeinschaft.

References

1. A. Messerschmidt and R. Wever, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 392–396.
2. W. Plass, *Angew. Chem. Int. Ed.* **1999**, *38*, 909–912.
3. A. Messerschmidt, L. Prade, and R. Wever, *Biol. Chem.* **1997**, *378*, 309–315.
4. W. Plass, A. Pohlmann, and H.-P. Yozgatli, *J. Inorg. Biochem.* **2000**, *80*, 181–183.
5. M. Bangesh and W. Plass, *J. Mol. Struct. THEOCHEM* **2005**, *725*, 163–175.
6. G. Zampella, P. Fantucci, V. L. Pecoraro, and L. De Gioia, *J. Am. Chem. Soc.* **2005**, *127*, 953–960.
7. S. Macedo-Ribeiro, W. Hemrika, R. Renirie, R. Wever, and A. Messerschmidt, *J. Biol. Inorg. Chem.* **1999**, *4*, 209–219.
8. G. J. Colpas, B. J. Hamstra, J. W. Kampf, and V. L. Pecoraro, *J. Am. Chem. Soc.* **1996**, *118*, 3469–3478.
9. M. J. Clague and A. Butler, *J. Am. Chem. Soc.* **1995**, *117*, 3415–3404.
10. G. E. Meister and A. Butler, *Inorg. Chem.* **1994**, *33*, 3269–3275.
11. A. F. Ghiron and R. C. Thompson, *Inorg. Chem.* **1990**, *29*, 4457–4461.
12. J. D. Lydon, L. M. Schwane, and R. C. Thompson, *Inorg. Chem.* **1987**, *26*, 2606–2612.
13. A. Butler and A. H. Baldwin, Structure and Bonding – Metal Sites in Proteins and Models; Springer, **1997**, pp. 111–132.
14. H. Vilter, *Phytochemistry* **1984**, *23*, 1387–1390.
15. ADF Version 2004-07, Scientific Computing & Modelling, Theoretical Chemistry, Virje University, Amsterdam, `http://www.scm.com`.
16. C. F. Guerra, J. Snijders, G. te Velde, and E. Baerends, *Theor. Chem. Acc.* **1998**, *99*, 391–403.
17. G. te Velde, F. M. Bickelhaupt, S. J. A. van Gisbergen, C. Fonesca, C. F. Guerra, E. J. Baerends, J. G. Snijders, and T. Ziegler, *J. Comput. Chem.* **2001**, *22*, 931–967.
18. R. Ahlrichs, M. Bar, M. Haser, H. Horn, and C. Kolmel, *Chem. Phys. Lett.* **1989**, *162*, 165–169.
19. A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098–3100.
20. J. P. Perdew, *Phys. Rev. B* **1986**, *33*, 8822–8824.
21. E. van Lenthe, E.-J. Baerends, and J. G. Snijders, *J. Chem. Phys.* **1994**, *101*, 9783–9792.
22. D. Andrae, U. Haeussermann, M. Dolg, H. Stoll, and H. Preuss, *Theor. Chim. Acta* **1990**, *77*, 123–141.
23. W. Plass, M. Bangesh, S. Nica, and A. Bucholz, *ACS Symposium Series* **2007**, *974*, 163–177.